estimated by studying the kinetics of molecular motors like helicases under different thermal conditions [1]. Here, we present some of the technical developments concerning a temperature-controlled Magnetic Tweezers setup that allows us to perform single-molecule experiments at temperatures in solution of up to 40 °C with a precision of 0.1 °C. Using this instrument we have been able to compare the translocation activity of individual copies of the bacterial DNA helicase-nuclease complex AddAB [2, 3] - an enzyme involved in the initial steps of double-stranded DNA break repair by homologous recombination - at different thermal settings with results obtained from ensemble measurements. Interestingly, although the two complementary approaches give rise to a systematic difference between their corresponding velocities measured at each temperature, they yield almost identical estimates of the kinetic barrier of the translocation process, which turns out to be on the order of 21 kT and hence similar to activation energies observed for other translocating proteins. Experiments to address the coupling between ATP hydrolysis and translocation by AddAB are ongoing and will also be discussed.

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1988-Pos Board B718

Single Molecule Studies of DNA-Binding Proteins: Development of New Covalent DNA Anchoring Techniques for the Study of Rupture Forces of Replication Blocks

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Magnetic Tweezers (MT) are a powerful technique to investigate the dynamics and kinetics of biomechanical processes *in-vitro* on a single-molecule level. In certain cases, it is necessary to apply unusually high pulling forces for the mechanochemical characterization of biomolecule structures and complexes, such as protein-nucleic acid complexes, DNA and RNA conformational overstretching conformation transition phenomena, or the unfolding of polymers and proteins at high resolution.

To achieve high pulling forces using MT, we have redesigned the anchoring strategy of biomolecules to the surface and magnetic bead, employing a bottom-up covalent chemisorption procedure. The development covers several aspects such as surface passivation to avoid aspecific physisorption, flexible and interchangeable covalent binding strategies using inert poly(ethylene glycol) linkers, and the characterization of chemical stability of tethered dsDNA sample constructs and overall coating density. Using this novel approach, dsDNA constructs have reproducibly and reliably withstood pulling forces of > 140 pN over long observation times.

We have applied our novel anchoring strategy to anchor DNA hairpins containing a single replication termination (*ter*) sequence. In *E.coli*, the DNA binding protein *Tus* binds to ter sites and is known to bring approaching replication forks to a halt by blocking strand separation. Upon opening our DNA hairpin in the presence of counter-helicase *Tus*, we mimicked replisome activity, and strand separation was demonstrably blocked. However, the barrier imposed was larger than our non-covalent anchoring method could withstand; it was impossible to 'break the lock'. Employing our new method, we are able to apply forces > 140 pN to the *Tus:ter* complex, allowing us to characterize the energy landscape of this system.

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Sensing the Association States of Single Biomolecules by Motion Analysis in an Electrokinetic Trap

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The different association states (i.e. bound/unbound, monomer/trimer, etc⋯) of a biomolecule are crucial to its function but yet difficult to differentiate at the single-molecule level. We describe a general approach to probe a single biomolecule's association states, and the time-dependent inter-conversion between these states in solution. Our approach is based on statistical analysis of molecular motions in an electrokinetic trap. This is possible because motion parameters (i.e. diffusion coefficient and electrokinetic mobility) are directly sensitive to intrinsic molecular properties of size and charge, which are modified as a molecule undergoes a change in association states. To accurately estimate single-molecule transport coefficients in solution, we employed photon-by-photon position tracking and developed an efficient algorithm based on Bayesian graphical models to analyze the raw tracking trajectories. As experimental demonstrations, we first used the method to resolve different populations in a multi-component mixture. Each measured single molecule, based on its size and charge characteristics reflected by transport properties, can be classified to a certain population. Differences as small as between the monomeric and trimeric forms of a fluorescent protein can be resolved. Second, to highlight the unique capability to probe time-dependent changes in molecular association states, we studied the hybridization kinetics of a 10-base ssDNA with its complementary partner. Digital, two-state, anticorrelated fluctuations in the measured diffusion coefficient and electrokinetic mobility were observed that directly sensed single-molecule binding and unbinding events in real time. We quantified the rates of the hybridization reaction by hidden Markov analysis of the time-dependent transport coefficients and investigated the destabilizing effect of a single-base mismatch.

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On-Chip Optical Manipulation of Biomolecule Arrays with Nm Resolution

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Optical trapping is a powerful manipulation and measurement technique widely employed in biological and materials sciences. Miniaturizing these instruments onto optofluidic platforms holds promise for high throughput lab-on-chip applications. However, a persistent challenge with existing optofluidic devices has been controlled and precise manipulation of trapped particles. Here we report a new class of on-chip optical trapping devices. Using on-chip optical interference functionalities, an array of ultra-stable, three-dimensional optical traps is formed by the evanescent field at the anti-nodes of a standing-wave in a nanophotonic waveguide. By employing the thermo-optic effect via integrated electric microheaters, the traps can be repositioned at high speed (~ 30 kHz) with nanometer precision. Using this device, we demonstrate trapping, sorting, and transport of particles, as well as manipulation of individual DNA molecules. Such a controllable trapping device has the potential for high-throughput precision measurements on chip.

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Pif1 Regulates Telomere Length by Removing Telomerase from Telomere Ends

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The repetitive TG-rich DNA sequence at the chromosome end, telomere, protects cells from the end-replication problem and abnormal DNA degradation in eukaryotic cells. The maintenance of telomere length is responsible for aging as well as cancer cell proliferation. Saccharomyces cerevisiae telomerase (Est2) is the reverse transcriptase responsible for extending telomeres in yeast. Pif1 helicase has been implicated in regulating the telomerase activity. We used single-molecule tethered particle motion experiments to directly investigate how Pif1 helicases regulate telomerase activity. We found that Est2 telomerase stayed bound to the telomere end after extension, but Pif1 helicases remove telomerase from the telomere. In the presence of Pif1 helicases, multiple runs of the telomerase-mediated telomere lengthening were observed. This suggests a model that Pif1 helicases remove telomerase from the telomere ends, allowing the telomerase recycling. We also observed that the ssDNA gap size affects the telomerase removal efficiency by Pif1 helicases, but not for the helicase unwinding efficiency. It is likely that efficient removal of telomerase from the telomere end is favored by the presence of multiple encountering of Pif1 helicases.

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3D Orbital Tracking of a DNA Locus during the Process of Transcription

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The temporal resolution currently achieved to track actively transcribing genes by means of fluorescence is of the order of 1-10s. We report on the application of 3D orbital tracking to a previously reported model system allowing to